REMARKS

Claims 1, 3-5, 8-9, and 12 were pending. Claims 71-101 were withdrawn by the examiner as related to an unelected invention and are canceled herein. Claim 4 is also canceled. Claims 1, 5, and 8 are amended. The amendment finds support throughout the specification at, *inter alia*, page 8, lines 22-24; Example 4; and the original claims. Claims 1, 3-5, 8-9, and 12 are pending. No claim is allowed.

Formal matters

The Examiner requested an update of all applications listed in the patent. The specification is amended herein accordingly to reflect any change in status of the cited applications.

Rejection Under 35 U.S.C. § 112, First Paragraph – Written Description

Claims 1, 3-5, 8-9 and 12 are rejected under 35 U.S.C. § 112, first paragraph as allegedly lacking written description. According to the examiner, there is no support in the specification as filed for the limitation "one or more agents" in claim 1. The Examiner also asserts that the specification fails to provide written description for the use of antibodies other than monoclonal antibodies. The Examiner asserts that the limitation "do not require IL-2" in IL-5 lacks support in the specification. The Examiner also argues that there is no support for the limitation "homogeneous population of Th2 cells comprises greater than about 50% Th2 cells." Applicants traverse these rejections.

Applicants respectfully submit that the specification as filed provides adequate support for the limitation "one or more agents" in claim 1. While the cited limitation is not disclosed *in haec verba* in the specification as filed, this alone is insufficient to render the limitation lacking in written description. *See, e.g., Purdue Pharma L.P. v. Faulding Inc.*, 230 F.2d 1320, 1323, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000). The agents of claim 1 are specifically delineated as IL-4, anti-IL-12 monoclonal antibody, and anti-IFNγ monoclonal antibody. Each of these agents is disclosed in the specification, and thus the use of this term does not broaden the scope of the claim beyond that which is supported by the specification.

The disclosure in the specification provides reasonable written description of the use of antibodies to various cytokines to modulate the T cell activation method claimed. However, in an

effort to expedite the prosecution of the application, the limitation "monoclonal" is included in the amended claims.

Applicants respectfully submit that the specification as filed provides adequate support for the limitation "do not require exogenous IL-2" in claim 5. The specification states at, *e.g.*, page 11, lines 23-25:

> The methods do not require use of IL-2. As a consequence, the expanded immune cells do not require IL-2 to retain activity or to remain viable.

The specification also states at page 8, lines 22-24 that the cells of the invention will not require exogenous IL-2. The cited disclosure in the specification provides adequate support for the cited limitation.

The claim encompassing the the limitation "homogeneous population of Th2 cells comprises greater than about 50% Th2 cells" has been canceled, rendering the rejection moot.

Accordingly, it is believed the bases for rejections may be withdrawn.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claim 4 is rejected under 35 U.S.C. § 112, second paragraph for alleged indefiniteness in the recitation of "homogenous". Applicant traverses this rejection.

The spelling of the recited term is corrected by amendment herein, rendering the rejection moot. Accordingly, it is believed this basis for rejection may be withdrawn.

Priority claim

The Examiner asserts that the claimed invention is not entitled to priority of the parent application Serial No. 60/044,693 because the specification allegedly contains no reference to a "homogeneous population of Th2 cells." The Examiner further alleges that the parent application contains no disclosure regarding the combination of agents in claim 1 (b) or the method of claims 4, 5, and 8. Applicant traverses this refusal to grant priority to the parent application.

Applicants respectfully submit the parent application fully supports the claimed methods, and therefore should be awarded the priority date of July 26, 1995. As a preliminary matter, Applicant notes that *in haec verba* disclosure is not required to fulfill the requirements under 35 U.S.C. § 112, first paragraph for a priority claim. *See* 37 C.F.R. § 1.51 (c). The cited provisional

application fulfills these requirements, and thus is properly a priority document for the claimed

methods.

The specification discloses methods to generate homogenous populations of Th2 cells.

The specification states beginning at page 6, line 23:

> Therefore, expanded use of ACI protocols will require technology that
> enables: the generation of <u>homogeneous</u> populations of effector immune
> cells; the consistent growth of effector cells to clinically relevant dosages
> (i.e., greater than 10^{10} cells) without the use of IL-2; a reduction in cost and
> labor to process cells; and the ability to reinfuse the cells without the need for
> systemic IL-2. ...
> The present invention addresses each of these requirements,
> disclosing a method to differentiate Th1 or Th2 cells *in vitro* and grow these
> cells to clinically relevant numbers without the use of IL-2. (emphasis added)

Applicants submit that this disclosure supports the claimed method to generate homogeneous

populations of Th2 cells as it states that the present invention addresses the requirement for the

homogeneous immune cells, and then cites the growth of Th2 cells in the following paragraph.

While not *in haec verba* disclosure of the limitation, a person of ordinary skill in the art would

recognize that the inventor had possession of the claimed method to generate homogeneous Th2

cells at the time of filing the provisional application in view of this disclosure.

The specification discloses the use of IL-4, the elected species, to generate Th2 cells.

For example, the specification discloses the use of IL-4 and anti-IFNγ monoclonal antibody to

generate to Th2 cells in Example 4 on page 36 of the specification of the provisional application.

Therefore, the provisional application supports the elected species of claim 1. While anti-IL12

monoclonal antibody is not expressly disclosed, Applicants submit that the disclosure cited above

sufficiently conveys to a person of skill in the art that anti-IL-12 could be used to generate Th2

cells, particulalry in light of the known effects of IL-12 in inducing IFNγ production.

Claim 4 is canceled herein, rendering this rejection moot.

The provisional application provides support throughout the specification for the method

of claim 5. For example, the specification states at page 12, line 7:

> For the purposes of the present invention, it is desirable to expand purified T-
> cells to clinically relevant numbers *in-vitro* without the use of exogenous IL-
> 2.

The specification also states that "the cells are not dependent on exogenous IL-2 for viability." See the specification of Serial No. 60/044,693 at page 8, lines 22-24. Therefore, the method of claim 5 is supported by the provisional application.

The specification of Serial No. 60/044,693 provides support for the method of claim 8. The monoclonal antibodies of claim 8 are found at, *inter alia*, page 12, line 6 to page 13, line 21. The specification also states that these monoclonal antibodies can be used in combination to provide the second signal to CD3 or CD2. *See, e.g.*, the specification of Serial No. 60/044,693 at page 13, lines 4-5.

Accordingly, Applicant respectfully submits that the the priority of the claimed invention should be the filing date of the parent application Serial No. 60/044,693.

Rejection Under 35 U.S.C. § 103 (a)

Claims 1, 3-5, 8-9, and 12 are rejected under 35 U.S.C. § 103 (a) as allegedly unpatentable over June et al., U.S. Patent No. 6,352,694 in view of Hsieh et al., and Cracauer et al., U.S. Patent No. 4,804,628. According to the Examiner, June et al. teaches that Th2 cells can be produced and expanded using the treatment of CD4+ T cells with anti-CD3 antibody and anti-CD28 antibody without the use of exogenous IL-2. The Examiner alleges that the CD4+ T cells can be antigen-specific, further isolated and purified, and expanded to reach greater than 10^{10} cells. The Examiner argues that June discloses the anti-CD3 and anti-CD28 antibodies are mitogenic and that starting material can be human T cells isolated from PBL. The Examiner acknowledges that June lacks any teaching regarding the specific concentration recited in claim 1 or the additional of exogenous IL-4. According to the Examiner, Cracauer teaches hollow fiber bioreactors and the use of the bioreactors to efficiently grow larger numbers of cells *in vitro*. The Examiner further alleges that Hsieh teaches that IL-4 increases Th2 in a concentration dependent manner. The Examiner argues that the skilled artisan would have been motivated combine the teachings of the cited references to make the claimed invention. Applicant traverses this rejection.

Applicant respectfully submits that June in view of Hsieh and Cracauer fails to render the claimed invention obvious because the cited combination fails to teach each and every element of the claimed methods. As acknowledged by the Examiner, June lacks any teaching regarding

expanding Th2 cells to 10^8 cells/ml as claimed. Neither Hsieh nor Cracauer remedy this deficiency. Hsieh lacks any disclosure regarding the generation of Th2 cells at this high concentration. Cracauer disclosure is limited to the expansion of hybridoma cells to high densities. *See, e.g.*, Cracauer at col. 5:57-66. Hybridoma cells are transformed lymphocytes created by cell fusion. *See, e.g.*, Exhibit A. Hybridoma cells are distinct from the normal lymphocytes of the claimed invention in that hybridoma cells are essentially tumor cells and thus are not sensitive to growth conditions and expansion limitations as the normal lymphocyte is. For example, hybridomas typically grow independently of cytokine or other stimulation. In other words, the ability to use a particular system to expand hybridoma cells fails to teach the use of normal lymphocytes or provide a reasonable expectation of success for expanding normal lymphocytes in that system. Therefore, the cited combination fails to render the claimed methods *prima facie* obvious because none of the cited references teach or suggest generating homogenous Th2 cells from normal lymphocytes at the claimed densities or a reasonable expectation of accomplishing such an expansion.

Accordingly, it is believed this basis for rejection may be withdrawn.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejections of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket No. 549172000113.

Dated: January 28, 2005

Respectfully submitted,

By _____

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regions of different isotypes have been prepared and shown to differ in strength of binding to polyvalent antigen (99). In the case of IgG3, the binding showed cooperativity. Thus the differences in antigen-binding strength due to the isotype of the constant region are postulated to be due to different contributions of Fc–Fc interactions.

MONOCLONAL ANTIBODIES

Homogeneous immunoglobulins have long played important roles in immunological research. Starting in the 1960s, human and animal myeloma proteins were studied as representative immunoglobulins and recognized for the advantages they had in studies of proteins as diverse as antibodies. Potter and colleagues characterized numerous mouse myeloma tumors and identified the antigenic specificities of some of them (100). These could then be used for studies of immunoglobulin structure, function, and genetics. It was not yet possible, however, to induce monoclonal immunoglobulins of desired specificity.

Production of antibodies of desired specificity in quantity and with reproducible characteristics had always been a challenge. These goals were achieved by the introduction of hybridoma technology by Köhler and Milstein (1,101) and by Margulies and colleagues (102) in the 1970s. Since that time, monoclonal antibodies have come to play an enormous role in biological research. They offer as advantages the relative ease of the production and purification of large quantities of antibody, the uniformity of antibody batches, and the ready availability of Ig mRNA and DNA from the hybrid cell lines.

Derivation of Hybridomas

This section presents an overview of the techniques of hybridoma derivation and a discussion of some of the issues involved. It will not attempt to provide a detailed, step-by-step protocol for laboratory use. For that purpose, the reader is referred to monographs and reviews on the subject (3,103–106).

Hybridomas producing monoclonal antibodies are generated by the somatic cell fusion of two cell types: antibody-producing cells from an immunized animal, which by themselves die in tissue culture in a relatively short time, and myeloma cells, which contribute their immortality in tissue culture to the hybrid cell. The myeloma cells are variants carrying drug selection markers so that only those myeloma cells that have fused with spleen cells providing the missing enzyme will survive under selective conditions. Successful hybridoma production is influenced by the characteristics of each of the

cell populations, the fusion conditions, and the subsequent selection and screening of the hybrids. Each of these areas will be discussed. A diagrammatic version of the overall process of hybridoma derivation is presented in Fig. 20.

Immunization of Donor Animals

Immunization protocols for fusion purposes have been developed empirically. A wide variety of standard routes and schedules of immunization can be used [e.g., antigen with complete Freund's adjuvant (CFA) subcutaneously or intraperitoneally and boosting with antigen in incomplete Freund's adjuvant or saline, or skin grafting and boosting with lymphocytes intraperitoneally], the main distinguishing feature being the use of a final intravenous boost with antigen 2 to 4 days before fusion. The importance and required timing of the intravenous boost are thought to be related to the type of cell that preferentially fuses (see below). Some investigators feel that animals should not be given the final boost and fused when they are at peak antibody titers, but rather should be rested until antibody levels decline, and then boosted for fusion (104).
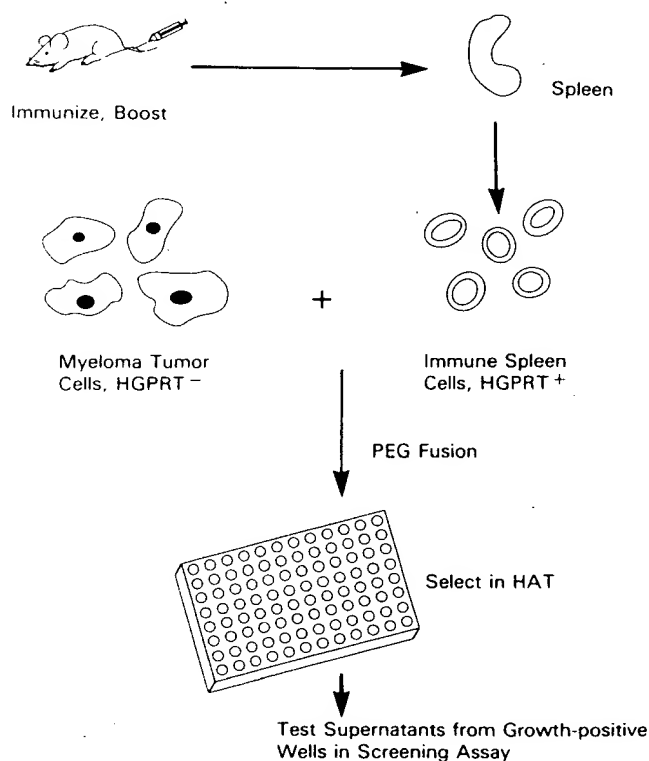

FIG. 20. Production of hybridomas. Steps in the derivation of hybridomas can be outlined as shown. Spleen cells from immunized donors are fused with myeloma cells bearing a selection marker (see Fig. 21). The fused cells are then cultured in selective medium until visible colonies grow, and their supernatants are then screened for antibody production.

Myeloma Cell Lines Used as Fusion Partners

One technical advance necessary for successful production of hybridomas was the development of drug-sensitive variants of myeloma cell lines. A drug marker is needed because the myeloma cells themselves will, of course, continue to proliferate in culture unless they are selectively killed to leave only the hybrids. A commonly used selective marker is sensitivity to medium containing hypoxanthine, aminopterin, and thymidine (HAT). The biochemical pathways involved are shown schematically in Fig. 21. Aminopterin poisons the *de novo* synthesis of purines. Spleen cells expressing the enzyme HGPRT necessary for the salvage pathway to recycle purines are able to survive in HAT medium but die after a short time unless immortalized by fusion with the myeloma cell. The myeloma cells, mutagenized and selected to be HGPRT-negative, are killed by the HAT-containing medium unless they have fused and therefore contain the enzymes of the spleen cell. Thus for several days after a fusion there is extensive cell death; subsequently, the culture should contain only cells resulting from spleen–myeloma fusion. Other drug markers are occasionally used, for example, ouabain resistance.

Initial work used myeloma cells, which retained the capacity to secrete their own immunoglobulin products (1). Later, such fusion partners were replaced by myeloma variants that express only one endogenous chain (101) or that fail to express Ig (107,108) so that the fused cell secretes primarily or exclusively antibody of the desired specificity. This confers a large advantage since, assuming random association, a cell making two heavy chains and two light chains would make Ig of which only $\frac{1}{16}$ was of the desired type.
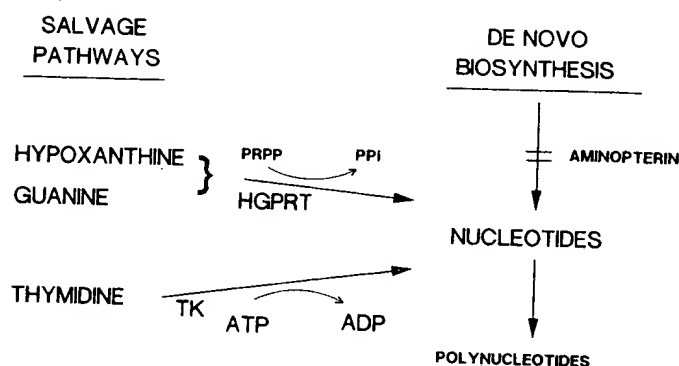
Myeloma lines for use in fusion have been further selected for desirable fusion properties. Franssen et al. (109) used the difficult approach of actual selection by fusion efficiency and identified a line that gave fusion efficiencies of one hybrid per 10^4 input spleen cells.

Periodically, the myeloma cells should be cycled through selective medium such as 8-azaguanine, to assure that they have not reverted to a drug-resistant phenotype, although this would be unnecessary for cell lines in which the mutation responsible for drug sensitivity is a deletion. Incorporation of 8-azaguanine into DNA is dependent on the salvage pathway, so it selectively kills HGPRT-positive cells.

Fusion Methods

Several different agents have been used to cause cell–cell fusion. Early somatic cell fusion work used Sendai virus (1). That approach has been replaced for routine fusions by use of polyethylene glycol (PEG). Both of these methods cause random fusions; many of the donor cells fusing will not be B cells in the right state to allow antibody production by the hybrid cell, and of those producing antibody, many will not make antibody of the desired specificity.

Typical efficiencies for successful PEG-mediated fusion are, for example, viable hybrid cells growing in 30% of the wells, with 5×10^4 cells plated per well. Much higher efficiencies are achieved on occasion. Factors appearing to influence efficiency strongly include the batches of PEG and of serum, such as fetal bovine serum used in the medium, and the subline of myeloma cells used as fusion partner. These batch-dependent factors unfortunately can only be tested by actual fusion procedures using any batches to be screened. Ability of fetal bovine serum, for example, to support growth of established hybridoma cells is not an adequate test of its ability to support fusion.

Of growth-positive wells, the proportion secreting antibody of the desired specificity is quite variable, depending presumably on the antigen, the screening assay, and the immunization protocol. The original descriptions of fusions producing anti-SRBC antibodies reported 10% specific antibody among viable hybrids (1), but many antigens give far lower yields than that. The poor yield of desired hybridomas from random fusion methods led some investigators to attempt selective fusion of those cells expressing receptors specific for a particular antigen.

Selective fusion methods involve attaching antigen to the myeloma cell, either directly or via an avidin–biotin bridge, for example, avidin-conjugated antigen added to biotin-coupled cells. Immune spleen cells are then mixed with the antigen-coated myeloma cells and cell aggregates are allowed to form. The aggregates are fused by addition of PEG (110) or by application of a strong elec-

FIG. 21. Pathways of nucleotide biosynthesis showing enzymatic steps that are altered in mutant cells used as fusion partners. Mutant cells lacking HGPRT or TK cannot use the corresponding salvage pathway for nucleotide biosynthesis. Such mutants cannot survive in medium containing aminopterin to poison the *de novo* synthesis pathway. However, individual mutant cells that have fused with spleen cells and thus do contain the HGPRT or TK enzyme can survive in appropriate selective medium by using the salvage pathway. HGPRT, hypoxanthine guanine phosphoribosyl transferase; TK, thymidine kinase.

tric field (111). Such techniques have been suggested not only to select for hybridomas of the desired specificity but also to favor fusion of cells making high-affinity antibody. However, the need for specialized apparatus has hindered the broad application of electrofusion, and antigen-focused fusion using PEG has not found wide acceptance.

Sample Protocol

A sample protocol for fusion in use in the laboratory of one of the authors follows. As stated above, this is not intended as a detailed laboratory guide, but rather as a description of the elements of the procedure.

Any time between day −14 and day −1: Prepare monolayers of peritoneal wash-out cells, 5×10^3 in 50 μl/well.

Day −3: Boost immunized mice intravenously with antigen.

Day −1: Split Sp2/0 myeloma cells, so that they will be in log phase on the day of fusion.

Day 0: Prepare and warm PEG. Prepare and count spleen cells and myeloma cells. Use 10 spleen cells per myeloma cell. Combine spleen cells and myeloma cells in a tube, pellet cells, and take off supernatant.

Add appropriate amount of 30% PEG and gently resuspend and mix during 2 min. Then spin for 6 min at 200g. Add 5 ml warm medium to dilute the PEG without disturbing the cell pellet. Spin for 5 min at 200g. Remove supernatant and resuspend cells gently in fusion medium. Dilute to the equivalent of 10^8 spleen cells per 60 ml, and plate out 100 μl/well onto peritoneal cell monolayers. As controls, plate out separately spleen cells and Sp2/0 cells that have not been fused, which should both die.

Day 1: Add 50 μl of 4× HAT to the fusion plates.

Day 7: Remove 100 μl of the supernatant and replace with fresh HAT-containing medium.

Day 10 and later: Watch plates for growth; test supernatants of growing wells for desired antibody.

Screening Methods

Methods for testing supernatants for desired antibodies can include the same range of methods used for studying such antibodies. Fusions have been successfully screened using RIA, ELISA, or other binding assays; visual immunofluorescence or flow cytometry; cytotoxicity assays; and assays for activation or blocking of biological effects such as cell-mediated lympholysis (CML), receptor activation, and lymphokine activity. Fusions can also be screened by hybridization to detect mRNA for Ig of certain types in the cells, rather than antibody in the supernatant (112).

The major issue in choosing a screening assay is that the assay not be subject to fluctuation, which would lead to many false-positive identifications and a large investment of effort in maintaining and cloning hybrid cells of no interest. Thus clear-cut discrimination between positives and negatives is often more important than exquisite sensitivity. Most supernatants contain at least several μg/ml of antibody, which is enough to detect by numerous methods.

A good screening assay should be convenient to use with hundreds of samples and should give results quickly, so that cells of greatest interest are still healthy when identified. If a parameter of interest is more difficult or time consuming to measure, it is often practical to use another assay for primary screening and then evaluate likely candidates in the more demanding assay. For example, a simple binding assay can be used for primary screening, and the positives then tested by immune precipitation to determine which bind to a particular component. Multiple pass screening is useful in many other situations in which two or more antibody characteristics are important in the choice of clones to keep.

A screening assay difficult to perform on very large numbers of samples can also be applied by testing supernatants pooled from small numbers of hybrids, provided the assay sensitivity would allow detection of one positive supernatant diluted in a pool of negative ones. Components of positive pools are then screened individually.

Postfusion Processing of Hybridomas

After identification of positive cultures, hybridomas must be cloned to assure production of only one antibody, and cells must be frozen for future use. Since a great deal of labor and material are consumed by processing of candidate hybridomas, an efficient strategy must be used.

It is often best to retest all hybrids before cloning any of them. Only those hybrids producing specific antibody again at the second screening are then cloned. Cloning can be performed by limiting dilution or by colony selection from soft agar. In either case it is best to clone promptly, before possible nonproducing cells in the same well, including variants of the positive cell, can overgrow the antibody producers. Newly derived hybridomas are often unstable in their antibody production, perhaps because somatic cell hybrids are aneuploid and throw off chromosomes. The uncloned lines are maintained until active clones have been well established. Clones producing desired antibody must then be expanded, supernatant collected for antibody preparation, cells frozen, and frozen cells thawed for verification of viability and antibody secretion.

Critical Factors in Successful Fusions

Establishment of fusion technology and production of desired monoclonal antibodies can be a frustrating process. Common problems include lot-to-lot variation in

PEG and fetal calf serum, requiring tedious batch testing. The characteristics of the myeloma cell used as fusion partner can also be a problem, since cell lines drift in their properties. The most efficient approach is to screen any new components and cells relative to ones that are in current use in another lab and are achieving good fusion efficiency. Another common problem is mycoplasma contamination of any of the cell lines or reagents used. All cell lines, including new hybrids once established, should be tested periodically for mycoplasma by available commercial kits or testing procedures.

Fusions in Species Other than Mice

Laboratory mice are the most common species immunized for hybridoma production, but for a variety of reasons other animal species often have advantages. If an antigen of interest is nonpolymorphic in the mouse, the mouse component might be immunogenic in other species, while mice would be tolerant to it. In the case of hybridomas for clinical use, mouse antibodies have the drawback of inducing anti-mouse immunoglobulin immune responses with possible deleterious effects, so derivation of human hybridomas is important.

Two approaches have been taken to the derivation of hybridomas in species other than mouse. First, interspecies hybridization can be performed. The resulting hybrids are often unstable and throw off chromosomes, but clones can sometimes be selected that produce antibody in a stable fashion. Examples of this would be rat–mouse fusion to produce antibody to the mouse Fc receptor (113), and hamster–mouse fusion to produce antibody to the mouse T3 equivalent (114). Rabbit–mouse hybridomas have also been described (115).

A second approach is the use of fusion partner cells from the desired species. Myeloma variants carrying drug selection markers are available in a number of species. A rat myeloma line adapted for this purpose, IR983F, was described by Bazin (116). This approach avoids some of the instability in interspecies hybrids and allows ascites production in homologous hosts.

Several approaches have been taken to the production of human monoclonal antibodies, and while none has been as successful as the production of mouse antibodies, some degree of success has been obtained. The major approaches are fusion of human cells with animal myelomas or with human tumor cells of various kinds (117,118), and use of Epstein–Barr virus to immortalize antibody-producing cells (119). Production of populations of sensitized human cells to be fused presents another special problem, since the donors cannot be immunized at will. In one example, *in vitro* stimulation of lymphocytes with antigen followed by fusion with mouse myeloma cells has been used to generate a series of antibodies to varicella zoster (120).

Another approach to production of monoclonal antibodies with human characteristics involves application of genetic engineering. When mouse monoclonal antibodies are used clinically, many of the complications are due to reactions to mouse Ig as a foreign protein, and thus there could be an advantage to minimizing the part of the antibody structure recognized as foreign by humans. Human constant regions can be combined with mouse variable regions (121,122) or even with just mouse hypervariable segments (see below, Applications) by molecular genetic techniques. The chimeric molecules may have many of the advantages of human hybridomas.

Cell Type from Immunized Donor that Fuses

The nature of the cell type from the immunized donor that actually fuses is not known, but some inferences have been made from indirect evidence. Köhler and Milstein found that the proportion of hybrids producing antibody specific for SRBC was high (averaging 10% of all viable hybrids). On this basis they suggested that most hybrids are derived from a small part of the total B cell population, a part including the antigen-stimulated cells (1,101). In a detailed study of the kinetics of the plaque-forming cell (pfc) response to antigen compared to results of fusions conducted at different time points, Paslay and Roozen (123) concluded that the peak hybridoma production preceded the peak pfc and the peak of serum antibody but corresponded to the peak of proliferation. These results point out that the success of a fusion does not necessarily correspond to the titers of sera drawn at the time of sacrificing the animals for fusion. It also helps explain the requirement for a final boost of antigen given approximately 3 days before fusion, which has been found advantageous by many investigators.

Applications of Monoclonal Antibodies

Since monoclonal antibodies can be made easily and reproducibly in large quantities, they allow many experiments that were not possible or practical before. They have advantages in many techniques such as immunofluorescence, ELISA or RIA, and immunohistochemistry because undesirable cross-reactions can be avoided. They have advantages for cell killing and for immunochemical separations because of the very high titers that can be achieved easily. Thus monoclonal antibodies are used in a wide variety of procedures, not only in immunology but also in other biological and physical sciences. This section gives only a sampling of such applications.

Affinity chromatography based on monoclonal antibodies can be used as a step in purification of molecular species that are difficult to purify chemically. Even a one-step purification can achieve enrichment adequate